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***Research Article** (Open Access)*

EVALUATION OF SELECTED BACTERIA FROM TEXTILE INDUSTRY EFFLUENTS FOR DYE DEGRADATION

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ABSTRACT
Kanchipuram and Tirupur are considered as the major textile industry hubs in Tamil Nadu and obviously most vulnerable places to environmental pollution because of the Xenobiotic compounds produced. 55 native bacterial strains were isolated from effluents, soil, and sludge collected from Kanchipuram and Tirupur region. The samples were analyzed for their physico-chemical parameters. Isolated strains screened for Malachite Green (MG) and Methylene Blue (MB) dye degradation by shake flask method. Based on the results, bacterial strains that showed higher activity on MG were SFT 3 and IEKC 10 and on MB were SFT 3 and IEKC 13 and hence chosen as the potential dye degrading strains for further studies. The present study signifies those native bacterial strains can be very well used for bioremediation of textile dyes.

Keywords: *Kanchipuram and Tirupur · Malachite green · Methylene Blue ·Bioremediation · Xenobiotic*

INTRODUCTION
Pollution caused by synthetic materials has a long-term impact on human health and galvanizing effects on socio-economic stability. Major among them are synthetic dyes, whose effluents that are left untreated or only partially treated are extremely toxic and pose a serious threat to the environment, particularly water and land resources (Vignesh *et al*., 2020). Proper disposal of such dye containing effluents are most challenging in the environmental protection point of view and lack of facilities and knowledge complicates it further. In dyeing processes, up to 15% of the dye applied does not adhere to the fibres and is released into the environment (Dauda and Erkurt, 2020). The World Bank anticipates about 20% of water contamination connected with textile production is caused by dyeing and treatment processes (Vikranta *et al*., 2018).
Due to textile industry's extensive water use (up to 150 l of water required to color 1 kg of cotton), they regularly produce enormous amounts of hazardous wastewater (Khan *et al*., 2018).
However, several countries do not abide by the effluent discharge norms due to their poor wastewater treatment facilities and ignorance of environmental issues. They quite often release an enormous quantity of incompletely or improperly treated effluents into the water bodies, which ultimately leads to serious environmental damage (Barathi *et al*., 2020). Over 100,000 synthetic textile dyes (7×10^7 metric tons), are produced annually, compromising the safety of the environment (Markandeya and Shukla, 2022). Henceforth, it is imperative to get rid of harmful substances before they are released into the environment. (Das and Mishra, 2017).
The effluent from the textile sector is characterized by its dark color, high pH, temperature, turbidity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), and total organic carbon (TOC) (Al-Tohamy *et al*., 2022; Kishor *et al*., 2021b). Intricate structures, very less biodegradability, and the presence of many xenobiotic compounds with high molecular weight, textile waste needs the most expensive and advanced processing methods which are not affordable to the developing nations (Vijayalakshmidevi and Muthukumar, 2015).
Azo dyes (R1-N=N-R2) are one of the oldest synthetic dyes structured with many possible substitutions which makes them extremely versatile and environmentally sustainable (Balapure *et al*., 2014). Methylene blue (MB) (3,7-bis(dimethylamino) phenothiazine chloride tetra methylthionine chloride, is a major

synthetic azo dye very commonly used in the coloring industries of silk, cotton, wool, and papers (Khodaie *et al*., 2013).

Triphenylmethane dyes are the next largest synthetic compounds used extensively across many coloring industries. Because of their complex structure of chromogen, which is composed of three phenyl groups bound to the central carbon atom, it's a tedious task to degrade these dyes from the effluents (Morales-Álvarez *et al*., 2018). Even though there are many dyes of this category, Malachite Green (MG) is the most popularly used one for coloring purposes (Rai *et al*., 2007).

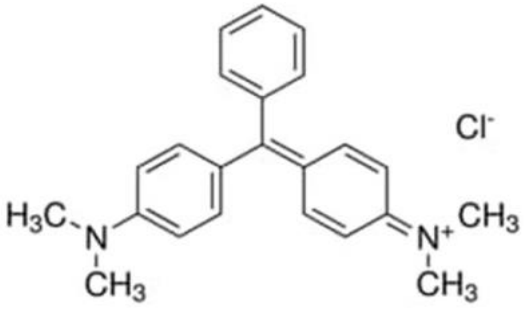
There are many conventional physico-chemical methods available to treat them which includes coagulation, flocculation, reverse osmosis technique, ozonization, oxidative and photochemical processes, ion exchange, and activated carbon adsorption yielding higher results. Those traditional methods despite their efficiency there are certain drawbacks which overrule them is their high costs and secondary sludge formation (Morales-Álvarez *et al*., 2018). Several microorganisms including bacteria and fungi isolated from contaminated zones possess the innate ability to decolorize or degrade the synthetic dyes and considered as the bio-weaponry against the dye-related environmental pollution (Song et al., 2020). Henceforth identifying these dye degrading/decolorizing microbes is of paramount priority in the present-day scenario (Du et al. 2011). Always there is a search to foster new ideas which could bring about an eco-friendly as well as budget-friendly method to treat the most toxic textile industry effluents. Focusing on the aforementioned ideas, this study aims in identifying potential bacterial strains for the degradation of two major dye varieties including MG and MB isolated from the textile industry effluents.

MATERIALS AND METHODS

Materials

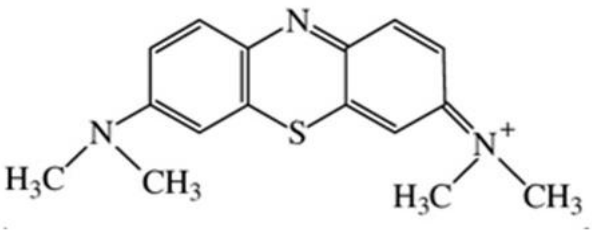
Chemicals and reagents **:** Malachite green (MG), Methylene blue (MB) and all other materials and reagents were generously provided by Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu.

Malachite Green (MG)



Group: Triphenyl methane dye
Chemical name: {4-[(4-dimethylaminophenyl)-phenyl methylidene]-dimethyl-ammonium chloride}
Molecular Formula: $C_{23}H_{25}ClN_2$
Molecular Weight: 364.9g/mol
λ max: 617nm

Methylene Blue (MB)



Group: Azo dye
Chemical name: 3,7-bis(dimethylamino) phenothiazin-5-ium as the counterion
Molecular Formula: $C_{16}H_{18}ClN_3S$
Molecular Weight: 319.9g/mol
λ max: 664nm

Nutrient Agar (NA) and Nutrient Broth (NB)

Methods

*Sample collection***:** Five Samples were collected from two different sites of Kanchipuram and Tirupur which are considered as the major textile industry hubs of Tamil Nadu state to analyze a broad spectrum of microbial community . As the above cities are major hubs for textile industries, water bodies in the vicinity of these cities are heavily polluted by the effluents of textile industries due to direct discharge into the environment or drainage system (Balakishnan *et al.,* 2008). Soil (sample 1) was collected from Kanchipuram textile industry dye contaminated zone in sterile containers and named as DCSK (Dye contaminated soil Kanchipuram). Effluent (sample 2) was collected from a Spectrum factory effluent line

and named as SFT (Spectrum factory Tirupur). Samples 3, 4, and 5 were collected from different spots of a common effluent treatment plant (CETP) of Kallikadu, Tirupur and named as IEKA, IEKB, and IEKC, respectively (Industrial effluent Kallikadu A, B, & C).

Analysis of the samples : The samples were collected and transported to the laboratory at 4°C by following the Standard methods for the examination of water and wastewater (APHA 2005). By using pH meter and laboratory thermometer, the pH and temperature were noted at the sampling site. Samples were transported to the lab within the minimum possible time to avoid further external microbial contamination and stored in cold storage to avoid further microbial growth. The physico-chemical parameters such as color, biological oxidation demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and total dissolved solids (TDS) were analyzed as described by Gupta (2004) as soon as the sample was brought to the laboratory.

Isolation of bacteria: Bacteria were isolated by standard spread plate method (Thomas *et al.*, 2012) using nutrient agar media prepared by adding nutrient agar (NA) with 100% distilled water amended with nystatin (100µg ml^{-1}), to inhibit the growth of fungi. All the five sample suspensions were serially diluted up to 10^{-6} dilutions using sterile distilled water blank. The pure bacterial colonies were identified and isolated. They were further sub-cultured on NA plates and stored at -20°C. Morphologically different bacterial colonies were selected and sub-cultured for further study.

Screening and Identification of potential Dye Decolorizing Bacteria : The selected isolates SFT 3, DCSK 10,IEKB 14,IEKC 10, and IEKC 13 were screened for their decolorizing ability to MG and MB dyes in two different concentrations (50 mg l^{-1} and 100 mg l^{-1}) in liquid culture medium (NB) by shake flask method (Vignesh *et al.,* 2020). After every 24h, 1ml of cell suspension was transferred to a 1.5ml Eppendorf tube and centrifuged for 10 minutes at 4500rpm for cells to settle. 0.1µl of each sample (control and treated) were slowly pipetted into micro titre plates and examined in UV Spectrophotometer (Biotek Epoch 2) as described by (Kalyani *et al.*, 2009) at 610 nm and 660 nm to measure its absorbance value. Readings were taken till partial or complete decolorization of broth was achieved. The decolorization rate was calculated by following the American Dye Manufacturing Institute ADMI tristimulus filter method (Kurade *et al*., 2012; Liu *et al*., 2018) and % decolorization was measured by following the equation as below:

ADMI removal (%) = (ADMIR0)− (ADMIRt)/(ADMIR0) × 100

where, ADMIR0 is the initial ADMI value and ADMIRt is the final value. In short, decolorization percentage was calculated by the formula:

% Degradation = A1 − A2/A1 × 100, where A1 is the initial absorbance, A2 is the final absorbance.

Identification of the potential isolates: By following Bergey's manual (1914) and Lee (2021) for the phenotypic methods of identifying the bacteria, microscopic and cultural characteristics of potential bacterial isolates were studied using NA media. By examining the cellular morphology (cell shape), staining characteristics (gram staining status), and growth characteristics (colony morphology) potential isolates were identified at the species level.

RESULTS AND DISCUSSION

Sample collection

Five samples were collected from two different textile dye contaminated sites in Kanchipuram (Latitude 11°01'44"N, longitude 77°19'44"E) and Tirupur (Latitude 11°08'44"N, longitude 77°34'07"E), Tamil Nadu (Fig 1). Kanchipuram is world famous for its silk-based textile industry which is the major reason for its water pollution (Kumar and Saravanan, 2015). Tirupur is well known for its dyeing and textile industry, because of its large-scale global production market they produce an enormous quantity of wastewater with high toxic content. These effluents were directly dumped into the environment, causing water and soil pollution (Sriram *et al.,* 2013). As described by Kumar and Saravanan about Kanchipuram and Sriram about Tirupur, samples were collected from the textile dye contaminated (most vulnerable) places itself.

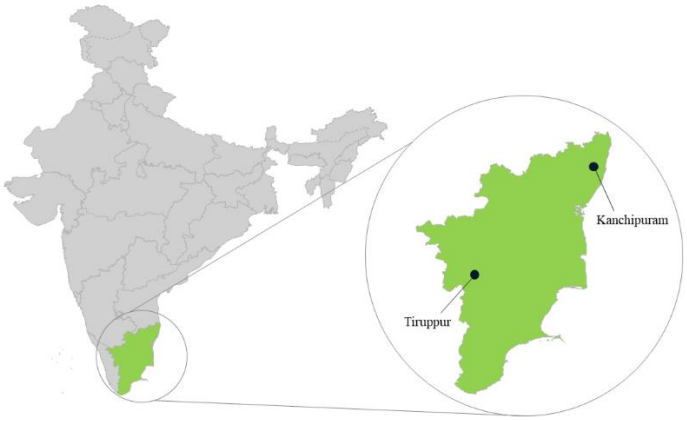


Fig.1 Sampling sites

Physico-chemical characterization of textile samples

The physico-chemical parameters of all the 5 samples such as color, pH, biological oxidation demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), total hardness and chloride levels were given in Table 1.

Table 1. Physico-chemical parameters of the 5 samples

	Samples				
Parameters	**SFT** (Effluent)	**DCSK** (Soil)	**IEKA** (Effluent)	**IEKB** (Sludge)	**IEKC** (Effluent)
pH	9	9.97	9.1	9.95	7.9
Biological oxygen demand (mg l^{-1})	245	NA	320	NA	40
Chemical oxygen demand (mg l^{-1})	788	NA	854	NA	115
Total suspended solids (mg l^{-1})	120	252	245	219	45
Total dissolved solids (mg l^{-1})	9858	401	9785	350	9245
Total hardness (mg l^{-1})	320	25	450	20	460
Color (Pt-Co)	1900	NA	2100	NA	NA
Chloride (mg l^{-1})	4500	695	5200	709	5300

The pH of all the five samples were alkaline. The alkaline nature of the water alters the properties of water and thereby affecting the life in it, including flora, fauna, and ultimately human beings. As explained by Shah (2013) in their work, effluent samples (from wastewater drain) collected in an industrial site at Ankleshwar Textile Industries, Gujarat, India were with high pH (alkaline) and high temperature. Further it was described that, alkalinity will affect the permeability of the neighbouring soil causing groundwater pollution and rise in temperature can bring down the soluble gases in water resulting in higher BOD and COD. The Biological Oxygen Demand (BOD) of the effluent samples collected were higher as 245 mg l^{-1}, 320 mg l^{-1} for SFT and IEKA and 40 mg l^{-1} for IEKC. The Chemical Oxygen Demand (COD) was noted as 788, 854 mg l^{-1} which was on the higher side and 115 mg l^{-1} for IEKC which was in general low. Total suspended solids (TSS) were noted as 120 mg l^{-1}, 252 mg l^{-1},245 mg l^{-1}, 219 mg l^{-1}, and 45 mg l^{-1}. Total dissolved solids (TDS) for all the effluent samples were almost same which was more than 9000 mg l^{-1}. TDS value has direct influence on sedimentation rate, which in turn will affect the penetration of light and ultimately photosynthesis (Delee *et al.*, 1998). In a recent literature (Kishor *et al.*, 2022), the COD, BOD, TSS, TDS, chloride level and few other parameters of textile industry's wastewater were compared with Central pollution control board values, which was found to be very higher in the untreated sample. Values were COD - 1746:250 (mg l^{-1}), BOD - 699 : 30 (mg l^{-1}), TSS - 501 : 100 (mg l^{-1}), TDS – 7203 : 2100 (mg l^{-1}) and chloride -1731:250 (mg l^{-1}). To analyse the toxicity level of textile effluents, above parameters can give a better outline about it.

Isolation of bacteria

It's a proven fact that textile effluent's native microbial diversity is a better choice for bioremediation of a wide variety of textile dyes because of their better adaptability and resistance to toxicity, which makes it an appropriate candidate for the decolorization of textile effluent studies (Samuciwal *et al.,* 2021). Totally, 55 morphologically different bacterial colonies were identified and isolated from the dye contaminated soil, textile industry effluents, and sludge. Out of which, 6 isolates were from Spectrum Factory Tirupur (SFT), 11 isolates from Dye contaminated soil Kanchipuram (DCSK), only 1 isolate from an Industrial effluent treatment plant, Kallikadu (IEKA), 24 isolates from Industrial effluent treatment plant, Kallikadu biological sludge (IEKB) and 13 isolates from Industrial effluent treatment plant, Kallikadu (IEKC). In a study conducted by Khan and Malik (2018), the number of microbial cultures were lesser in wastewater and dye contaminated soil than the counts in the ground water containing soil. The microorganisms isolated from the effluent sample collected from the industrial site (Ankleshwar Textile Industries, Gujarat) was fool proof evidence for the adaptability efficiency of the microbes to exist in the more toxic synthetic chemicals and dyes (Shah 2013). Likewise, Olukanni *et al.* (2006) demonstrated the better effect of 18 adaptive bacterial strains in effluent treatment out of the 24 varieties isolated, including both adaptive (isolated from textile effluents) and non-adaptive (isolated from land-fill sites).

Screening and Identification of potential Dye Decolorizing Bacteria

The selected isolates SFT 3, DCSK 10, IEKB 14, IEKC 10 and IEKC 13 were screened in the liquid media (NB) supplemented with MG and MB dyes. The test procedures were done as per regular protocol, after every 24h of incubation period, samples checked for its degrading efficiency under UV spectrophotometer in the absorbance value of 610nm and 660nm for MG and MB respectively.

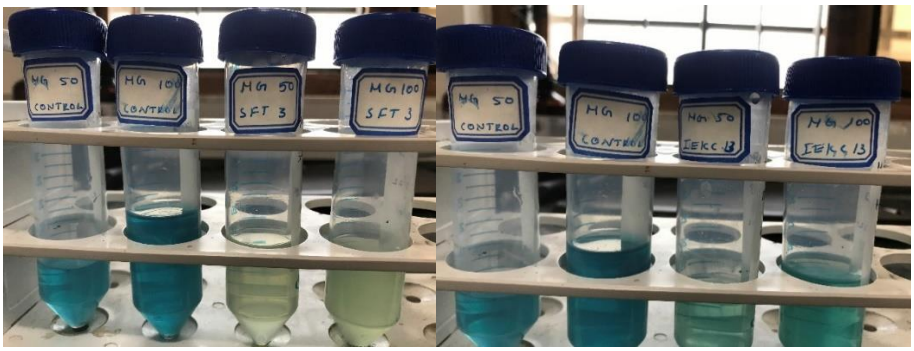


Fig 2: Decolorization of MG. A - treated with SFT 3. B - Treated with IEKC 13.

Isolate SFT 3 started giving results for MG within 24 h of incubation period with 61.83% in 50 mg l^{-1} and 69.80% in 100 mg l^{-1} decolorizing rate (Fig.2). The maximum activity of 90% and above was attained by SFT 3 at 120h interval in both the concentrations. Similarly, isolate IEKC 10 showed maximum degradation rate of 90.83% in 50 mg l^{-1} and 90.61% in 100 mg l^{-1} dye concentration in 216h and 120h, respectively. Even though the strain DCSK 10 exhibited more than 90% decolorizing rate within 168 h and 216 h with 50 mg l^{-1} dye concentration, the results were not promising with 100 mg l^{-1} the dye concentration. The results were plotted in Fig.3 A and B. Similar observation was reported during MG decolorization by *Sphingomonas paucimobilis* isolated from the soil sample collected from the textile industry contaminated sites of KsarHellal, Tunisia, in which 50 mg l^{-1} concentration within 4 h under shaking condition and the degrading capacity increased with the increase in biomass (Ayed *et al.,* 2009). Furthermore, the results of Chaturvedi and Verma (2015) explains the native microbes' efficiency in MG degradation by the activity of *Ochrobactrum pseudogrignonense* strain GGUPV1. This strain GGUPV1 isolated from the wastewater of copper mine was able to show maximum activity (degrade 400 mg l^{-1}) in minimal media and in the presence of copper sulphate (10 and 20 mM) was also able to degrade at 100 mg l^{-1} of MG.

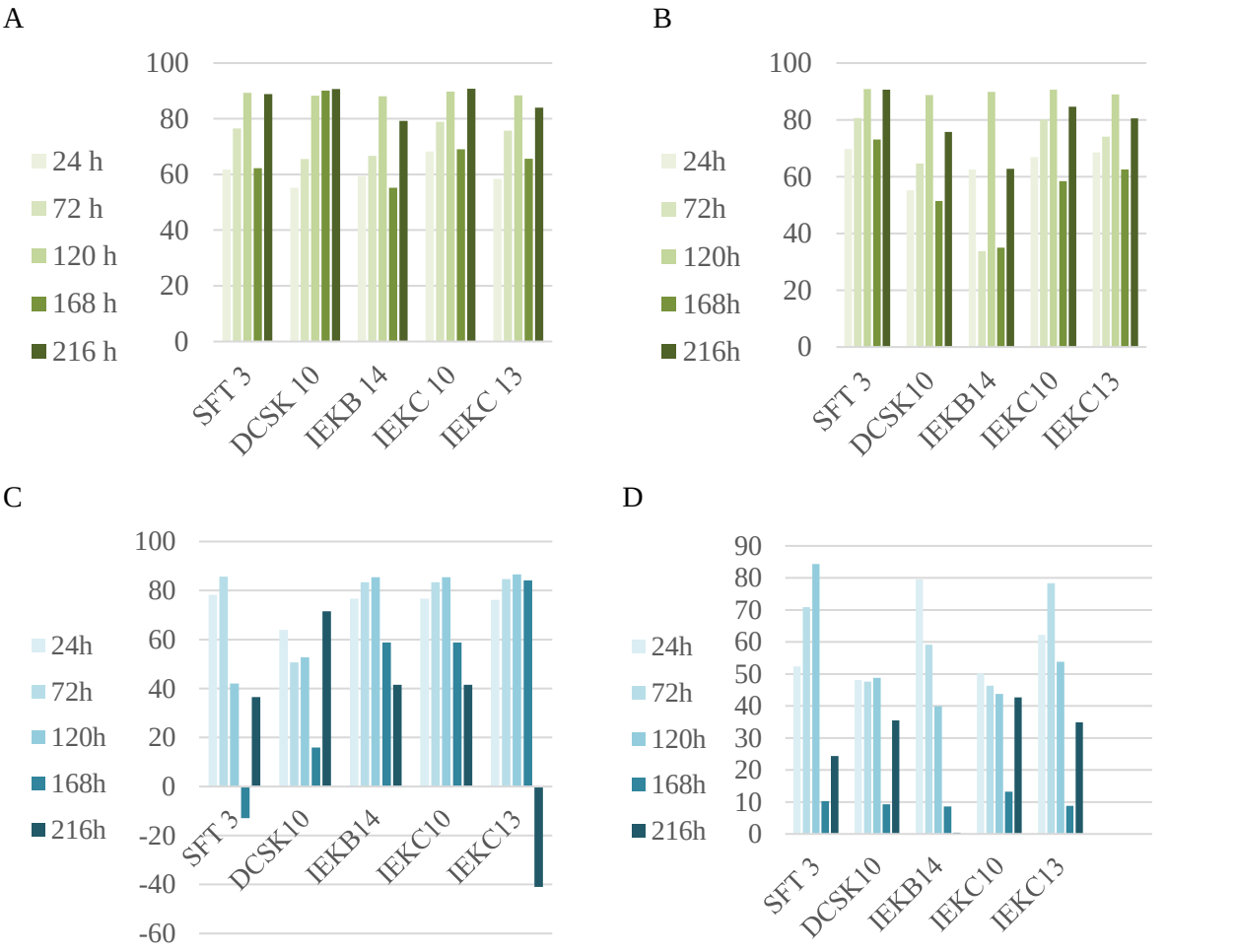


Fig 3: Decolorization of dyes in NB. A - MG 50 mg l^{-1} concentration. B - MG 100 mg l^{-1} concentration. C - MB 50 mg l^{-1} concentration. D - MB 100 mg l^{-1} concentration

In MB degradation studies, out of the 5 selected isolates, isolate SFT 3 decolorized 85.71% at 50 mg l^{-1} concentration of MB within 72 h and 84.34% at 100 mg l^{-1} within 120 h of the incubation period. Similarly, isolate IEKC 13 showed a maximum degradation rate of 86.65% at 50 mg l^{-1} within 120 h and 78.34% at 100 mg l^{-1} within 72 h of the incubation period when compared to the other isolates.

Interestingly in both isolates, the activity slowly started showing decreasing rate with an increase in the incubation time. This may be due to the reason that the bacterial population could have used the nutrients available and was not able to grow further and continue with degradation activity. The results were given in Fig.3 C and D. These results build on existing evidence with *Serratia marcescens* (isolated from only one soil sample) that revealed good results for the decolorization of Biebrich scarlet and Direct Blue 71, two azo dyes, by the stab-culture method out of the twenty soil samples tested (Syed *et al.*, 2009). Similarly, in an experiment conducted with the direct azo dyes, Orange 3R, Blue 3R, Yellow Gr, Black RL, and T blue, *Pseudomonas fluorescens* was most effective, next by Bacillus sp., and *Escherichia coli*, and proved to be the potential bioremediation source for the effluent treatment (Manivannan *et al.*, 2011).

Based on the screening, bacterial strains that showed the highest activity on MG were SFT 3 and IEKC 10 and upon MB were SFT 3 and IEKC 13. Henceforth, chosen as the potential dye degrading isolates for the research work further.

Identification of the potential isolates

Under microscopic observation, phenotypic characteristics such as cultural and micromorphology revealed that the strains SFT3 found to be *Staphylococcus sp*., IEKC10 as *Stappia sp*., IEKC13 and DCSK10 as *Bacillus sp*., and IEKB14 as *Pseudomonas sp*.

Table 2. Phenotypic characteristics and identification of isolates

Strains	Gram staining	Shape	Colour	Colony	Identified as
SFT3	Gram +ve	Spherical	White	Clusters	*Staphylococcus sp.*
DCSK10	Gram +ve	Rod shaped	Half white	Rough, dry and irregular	*Bacillus sp.*
IEKB14	Gram -ve	Rod shaped	Greenish	Swarming colony	*Pseudomonas sp.*
IEKC10	Gram -ve	Rod shaped	Tan colour	Circular smooth colony	*Stappia sp.*
IEKC13	Gram +ve	Rod shaped	Half white	Rough, dry and irregular	*Bacillus sp.*

CONCLUSION

Textile industry's effluent treatment even though is an arduous process, if left unattended can affect the health and wealth of any country. Since conventional wastewater treatment methods involves more cost, time, and toxic chemicals, once again it becomes a cause for secondary pollution. So always there is a need for a better and eco-friendly procedure to control this global problem. Based on the previously available literatures and the results of this research, it is suggested that the native bacterial strains SFT 3, IEKC 10, and IEKC 13 have greater potential towards the biotreatment of textile effluents and could be further confirmed by doing UV-Vis spectroscopy, FT IR, GC MS and Toxicity analysis for the potential strains in the further research.

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CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

Abbreviations: *MG - Malachite green, MB - Methylene blue, NA – Nutrient agar, NB – Nutrient broth.*

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